Naked DNA in cells: An inducer of major histocompatibility complex molecules to evoke autoimmune responses?

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Abstract

The major histocompatibility complex (MHC) is the exclusive chaperone that presents intracellular antigens, either self or foreign to T cells. Interestingly, aberrant expression of MHC molecules has been reported in various autoimmune target tissues such as thyroid follicular cells in Grave's disease. Herein, we review the discovery of an unexpected effect of cytosolic double-stranded DNA (dsDNA), despite its origins, to induce antigen processing and presenting genes, including MHC molecules, in non-immune cells. Moreover, we highlight several recent studies that suggest cell injury endows thyroid epithelial cells with a phenotype of mature antigen presenting cells by inducing multiple antigen processing and presenting genes *via* releasing genomic DNA fragments into the cytosol. We discuss the possibility that such cytosolic dsDNA, in naked form without binding to histone proteins, might be involved in the development of cell damage-triggered autoimmune responses. We also discuss the possible molecular mechanism by which cytosolic dsDNA can induce MHC molecules. It is reasonable to speculate that cytosolic dsDNA-induced MHC class I is partially due to an autocrine/paracrine effect of type I interferon (IFN). While the mechanism of cytosolic dsDNA-induced MHC class II expression appears, at least partially, distinct from that mediated by IFN-γ. Further in-depth are required to clarify this picture.

Key words: Cytosolic double-stranded DNA; Major histocompatibility complex molecules; Autoimmune response; Antigen presentation; Tissue injury

Core tip: We reviewed the discovery of an unexpected effect of cytosolic double-stranded DNA (dsDNA) to induce antigen processing and presenting genes including major histocompatibility complex (MHC) molecules in non-immune cells. We also focus on the

current status quo of the overall research in the field with highlight on our recent findings that suggest cell injury-induced self-cytosolic dsDNA is a potential trigger in the development of autoimmunity. Meanwhile, we provide in-depth discussion of the molecular signals responsible for the effect of dsDNA to induce MHC molecules, based on the current opinion of dsDNA-mediated signal pathways.

INTRODUCTION

The major histocompatibility complex (MHC) is the exclusive chaperone that presents intracellular antigens (more specifically, peptides), either synthesized by the cell itself or internalized from the extracellular environment, to T cells. Upon binding to the MHC-peptide complex *via* the T cell receptor and CD4/CD8 co-receptors, T cells should in principle tolerate self antigens. In contrast, activation should occur if the T cells had not been trained to recognize the antigen during thymus positive selection. If this principle, known as immune tolerance, is violated, an autoimmune response will occur. Interestingly, aberrant expression of MHC molecules has been reported in endocrine epithelial cells in autoimmune target tissues (Table 1) such as pancreatic beta cells of insulin-dependent diabetes[1] and thyroid follicular cells of Grave's disease[2-5]. Transgenic mouse strains harboring MHC linked to insulin promoter overexpress MHC molecules in pancreatic beta cells and spontaneously develop insulin-dependent diabetes[6-8]. Many attempts have been made to artificially induce Grave's disease in mice. Although not very successful, these efforts have demonstrated that autoantibodies developed only when the animals were immunized with cells co-expressing the self antigen, thyroid stimulating hormone receptor (TSHR), and MHC class Ⅱ molecules[9]. The few successful mouse models of Grave's disease were generated by vaccination of TSHR-expressing plasmids or by infection of TSHR-expressing adenovirus[10,11]. Significantly, both DNA vaccine and adenovirus are primarily double-stranded DNAs (dsDNAs) that should be able to induce MHC molecules in the cells at the site of inoculation. Does this "adjuvant-effect" contribute to the successful generation of the autoimmune mouse model?

CYTOSOLIC DOUBLE-STRANDED DNA INDUCES MHC MOLECULES IN NON-IMMUNE CELLS

In 1999, Suzuki *et al*[12] reported that the expression of MHC molecules, including MHC class Ⅰ and MHC class Ⅱ, could be strongly induced by the transfection of dsDNA, regardless of their origins. Diverse dsDNAs were tested *in vitro,* including bacterial DNA, viral DNA, salmon sperm DNA, calf thymus DNA, host genomic DNA, plasmid DNA and artificially synthesized DNA fragments. Remarkably, all induced MHC expression, whereas single-stranded DNA (ssDNA) could not[12]. To exert this effect, dsDNA needs to be introduced into the cytosol, as free dsDNA in extracellular medium was not sufficient to induce MHC expression[13], indicating that this effect is unlikely mediated by cell surface receptors. The method of introducing dsDNA into cytosol is not critical. Different transfection procedures, including lipofection, electroporation and diethylaminoethyl-dextran similarly increased MHC levels[12]. The first study that explicitly and thoroughly described such effects specific to cytosolic dsDNA was not reported until 1999. This was surprisingly late, considering that cell culture transfection methods were developed in the 1970s and became widespread during the 1980s, although it had been previously observed that fibroblasts could somehow respond to nucleic-acids derived from pathogens or the host[14,15].

The effect of cytosolic dsDNA does not require professional antigen presenting cells (APCs). In addition to professional APCs, including primary cultures of mouse dendritic cells (DCs) and macrophages, the induction of MHC molecules by cytosolic dsDNA was reproduced in non-professional APCs such as rat thyroid follicular cells, human and mouse fibroblasts, human and mouse muscle cells and human endothelial cells[12,13,16,17]. These results imply the possibility that a potential APC pool consisting of various non-immune cells *in vivo* can be activated upon their exposure to cytosolic dsDNA (possibly derived from invasive pathogens or dying host cells). In particular, direct evidence has shown that MHC-expressing thyroid epithelial cells are potentially competent APCs to present antigens to activate T cells. MHC class Ⅱ-positive human thyroid follicular cells were able to present a influenza-specific peptide to a human T-cell clone, a reaction which was abrogated by anti-MHC class Ⅱ antibodies[3]. Lectin-induced MHC class Ⅱ-positive human thyroid cells in monolayer culture were able to induce a proliferative reaction in autologous T cells, a phenomenon not found with MHC class Ⅱ-negative cells[18]. Wistar rats are susceptible to the induction of experimental autoimmune thyroiditis. A cloned Wistar thyroid epithelial cell line was shown to be directly recognized by Wistar rat lymphoid T cells that were both MHC class Ⅰ- and class Ⅱ-restricted[19]. When CBA mouse lymphoblasts generated on co-culture with monolayer syngeneic thyroid epithelial cells were injected either intravenously or into the thyroid lobes of intact CBA recipients, thyroiditis appeared within three weeks[20]. All these evidence suggest that the potential ability of thyroid epithelial cells as APCs to directly interact with T cells in a MHC-restricted manner likely precipitates autoimmune response in the thyroid, although whether exposure to cytosolic dsDNA would

Table 1 Inappropriate expression of major histocompatibility complex molecules in autoimmune disorders

Disease	Cells with aberrant expression of MHC molecules
Insulin-dependent diabetes	Pancreatic beta cells[1]
Grave's disease	Thyroid epithelial cells[54]
Rheumatic carditis	Valvular fibroblasts[55]
Primary biliary cirrhosis	Bile duct epithelial cells[56]
Sjögren's syndrome	Salivary acinar and ductal epithelial cells[57]
Acute lymphoproliferative disorders	Bone marrow-derived mesenchymal stromal cells[58]
Asthma[1]	Bronchial epithelial cells[59]
Dilated cardiomyopathy[1]	Endothelial and endocardial cells[60]
Tubulointerstitial nephritis[1]	Renal tubular epithelial cells[61]
Biliary atresia[1]	Intrahepatic bile ducts[62]

[1]A role of autoimmunity is suggested in the pathology of the indicated diseases. MHC: Major histocompatibility complex.

substantialize such potential in non-immunes cells needs to be further tested by experiments.

Unmethylated CpG motifs, which commonly exist within bacterial DNA and viral DNA, but not in vertebrates, have been shown to activate innate immunity *via* CpG sensor toll-like receptor 9 (TLR9)[21]. However, CpG motif-containing ssDNA failed to induce MHC molecules whereas methylase-treated CpG dsDNA induced MHC expressions to a level comparable to untreated CpG dsDNA[12]. These results indicated that the induction of MHC molecules by cytosolic dsDNA is not mediated by CpG motifs. In contrast, DNase treatment, as predicted, completely abolished the induction of MHC molecules following dsDNA transfection[12,13]. Later, it was shown that the effect of cytosolic dsDNA to stimulate a host innate immune response was independent of TLRs, but required a classic double-stranded right-handed helix sense (B-DNA)[22] with a native sugar-phosphate backbone[16]. Although the effect of dsDNA on MHC appears sequence-independent, MHC expression was shown to be induced by dsDNA in a length-dependent manner[12,13]. Nevertheless, a DNA fragment as short as 25 bp was capable of exerting a reproducible concentration-dependent effect on the expression of MHC molecules[12]. It was shown in later studies that cytosolic dsDNA activated innate immune responses in a length-dependent manner. This result might indicate an increasing binding affinity by putative cytosolic dsDNA sensors for longer dsDNA[22-24].

Antigen processing and presenting is a multiple-step process involving numerous molecules with diverse functions, such as proteasome proteins responsible for antigen degradation to generate peptides, *e.g.*, LMP2 and cathepsin; molecules required for transporting and loading peptides onto MHC molecules, *e.g.*, transporter associated with antigen processing, MHC Ⅱ-associated invariant chain (Ii), and HLA-DMB; cell surface co-stimulators indispensable for fully activating T cells, *e.g.*, CD80, CD86, CD40; and cell adhesion molecules for stabilizing the binding with lymphocytes, *e.g.*, CD54 (also known as intracellular adhesion molecule 1)[25,26]. In addition to MHC molecules, many of these essential participants in antigen processing and presenting, as well as the transcription factors for MHC expression, including signal transducers and activators, interferon regulatory factor 1, nuclear factor κB (NF-κB), and class Ⅱ MHC transactivator (CⅡTA), have also been shown to be induced/activated by cytosolic dsDNA, but not ssDNA, in both professional APCs and non-professional APCs[12,13,16,17]. Overall, these results suggest that in the presence of cytosolic dsDNA, even non-immune cells can acquire full capability to present antigens (so called APC maturation). Theoretically, T cells should have a much greater chance to be activated by antigens under this condition. In agreement with such a prediction, production of interleukin-2 (IL-2) and interferon-γ (IFN-γ) in T cells was significantly increased (approximately 3-fold) by mixing with peptide-challenged DCs containing cytosolic dsDNA, compared to those without cytosolic dsDNA, or those containing cytosolic ssDNA[13].

TISSUE INJURY INDUCES APC MATURATION *VIA* CYTOSOLIC DNA

DNA, normally sequestered within the nucleus or in mitochondria, can be internalized into the cytosol of phagocytes from apoptotic bodies released from dying cells *in vivo*. Phagocytes engulf the apoptotic bodies (also the nuclei expelled from erythroid precursor cells) in extracellular medium and digest the DNA contents using DNase in the phagolysosomes[27,28]. Indeed, a large amount of cytosolic DNA has been demonstrated to accumulate in DNase-deficient murine macrophages that were presented apoptotic cells, but only a small amount in wild-type macrophages after the same treatment[29]. Defective clearance of self DNA due to mutations in DNase genes is associated with the development of human autoimmune diseases such as systemic lupus erythematosus[30,31] and Aicardi-Goutieres syndrome[32,33], thus revealing self DNA as a potential trigger for autoimmune responses.

Despite the presence of normal DNase, tissue injury, depending on the severity, can generate more dying cells than the intrinsic digestive capacity of phagocytes can process, inevitably leading to cytosolic DNA accumulation in phagocytes. It has been shown that electric pulse-mediated cell injury under sterile conditions induced cytosolic DNA in a current intensity-dependent manner in rat thyroid epithelial cells[17], supporting the notion that tissue injury is a possible cause for the presence of cytosolic DNA *in vivo*. Intriguingly, electric pulse-caused cell injury also induced the expression of MHC Ⅱ and its transactivator CⅡTA in a current intensity-dependent manner in rat thyroid epithelial cells[12]. Such cell injury endows thyroid epithelial cells with a phenotype of
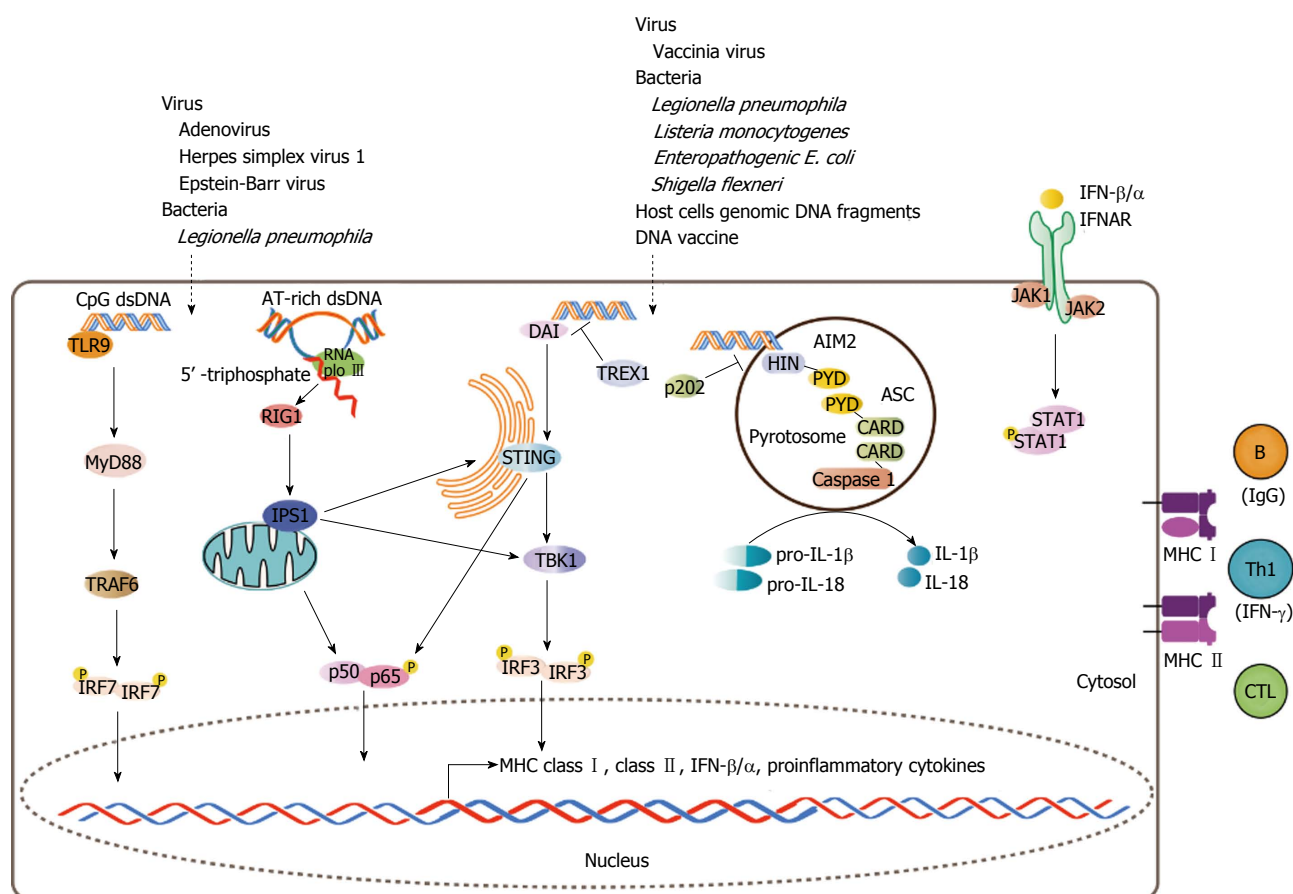
Figure 1 Cytosolic double-stranded DNA signal pathways. TLR9-dependent and TLR9-independent signal pathways have been proposed to mediate foreign- or self-derived cytosolic dsDNA signal to induce the expression of MHC molecules, type Ⅰ IFNs, and proinflammatory cytokines. At the same time, cytosolic dsDNA can trigger AIM2-mediated inflammasome formation to produce active IL-1 and IL-18. Consequently, exposure to cytosol dsDNA will increase the probability of (auto)immune response. TLR9: Toll-like receptor 9; MyD88: Myeloid differentiation primary response gene 88; TRAF6: Tumor necrosis factor receptor associated factor 6; IRF7/3: Interferon regulatory factor 7/3; RNA pol Ⅲ: RNA polymerase Ⅲ; RIG1: Retinoic acid-inducible gene 1; IPS1: Interferon-promoter stimulator 1; DAI: DNA-dependent activator of interferon - regulatory factors; TREX1: 3-5 exonuclease (also known as DNase Ⅲ); STING: Stimulator of interferon genes; TBK1: TANK-binding kinase 1; AIM2: Absent in melanoma 2; ASC: Apoptosis-associated speck-like protein complex; HIN: C-terminal HIN-200 domain; PYD: N-terminal pyrin domain; CARD: Caspase activation and recruitment domain; IFNAR: Type Ⅰ interferon receptor; STAT1: Signal transducer and activator of transcription 1; JAK1: Janus kinase 1; CTL: Cytotoxic T lymphocytes; IFN: Interferon; MHC: Major histocompatibility complex; IL: Interleukin; dsDNA: Double-stranded DNA.

mature APC by inducing multiple antigen processing and presenting genes that largely overlapped with cytosolic DNA-inducible molecules such as MHC Ⅰ, MHC Ⅱ, CD40, CD54, CD80, CD86, RFX5 and CⅡTA[12,17].

APC maturation characterized by increased expression of CD40 was also observed in primary cultures of immature murine CD11c+ bone marrow dendritic cells (BMDCs) when cultured with necrotic fibroblasts derived from the same animal[13]. Proteinase-K-treated necrotic fibroblasts reproduced the same effect, inducing APC maturation in BMDCs to a similar extent[13], indicating self protein was unlikely the essential factor. However, additional DNase treatment significantly abrogated the ability of necrotic fibroblasts to induce APC maturation[13], suggesting it is likely that the self DNA derived from the dying cells had contributed to the activation of APCs. However, the profile and kinetics of cell injury-induced genes was not completely duplicated by that induced by dsDNA transfection[12,17], indicating additional factors other than cytosolic DNA may be involved in a cell injury event.

MOLECULAR SIGNALS FOR CYTOSOLIC DNA-INDUCED MHC MOLECULES

Cytosolic DNA-mediated signal pathways have been extensively investigated (well-reviewed in[34]). Two independent responses can be simultaneously induced by cytosolic dsDNA, one characterized by the production of type Ⅰ IFNs (IFN-α and -β) as well as type Ⅰ IFN-inducible molecules, and a second pro-inflammatory response characterized by the production of IL-1β and IL-18, both of which have been implicated in the activation of the immune response[34] (Figure 1).

It is reasonable to speculate that cytosolic dsDNA-induced APC maturation is partially due to an autocrine/paracrine effect involving secreted type Ⅰ IFNs, mediated by the cell surface type Ⅰ IFN receptor (IFNAR) (Figure 1). Such a process could stimulate APC maturation in DCs and precipitate T cell activation *in vitro* and *in vivo*, concomitantly with increased expression of antigen processing and presenting genes, including MHC Ⅰ, CD40 and CD86[35-37] (Figure 1). Repeated low-

dose chemotherapy or radiation could also trigger an autoimmune response. These cellular insults can induce MHC Ⅰ expression in cancer cells *via* the IFN-β/IFNAR signal pathway[38] and enhance CD8^{+} T cell-mediated antitumor immune responses to tumor vaccine *in vivo*[39]. As a therapeutic strategy to restore autoimmune surveillance in cancer cells, low-dose chemotherapy is given to metastatic pancreatic cancer patients before receiving a cell-based cancer vaccine[40]. Thus, cell damage-induced autoimmunity may not be entirely harmful if wisely used.

Moreover, it is possible that the induction of type Ⅰ IFNs and antigen processing and presenting genes share some upstream signals in common, such as STAT and NF-κB signal pathways that could be directly activated upon the recognition of cytosolic dsDNA as a "danger signal"[12,17,34,41]. Thus, it is likely that the signal pathways that mediate type Ⅰ IFN production and the induction of antigen processing and presenting genes cross-talk with one another (Figure 1). Stimulator of IFN genes (STING)[42,43] and TANK-binding kinase 1 (TBK1)[44,45], which have been shown to mediate cytosolic dsDNA-induced type Ⅰ IFN production (Figure 1), may also be required for the induction of antigen processing and presenting genes by cytosolic dsDNA, as DNA vaccine-mediated T cell activation was abolished in STING-knockout mice that were challenged with antigen peptides[43]. Moreover, TBK1-deficiency abrogated cytosolic dsDNA-induced APC maturation in primary mouse bone marrow macrophages[45]. Further studies are required to clarify this picture.

Cytosolic dsDNA-induced MHC Ⅱ expression should be IFNAR-independent, as type Ⅰ IFNs do not induce MHC Ⅱ, but rather suppress its expression by acting as an antagonist of IFN-γ[46], especially in non-professional APCs[47]. Indeed, cytosolic dsDNA prominently induced MHC Ⅰ rather than MHC Ⅱ in rat thyroid epithelial cells *in vitro*[12]. Knockout mice have revealed something more *in vivo*. Both MHC Ⅰ and MHC Ⅱ induction occurred in areas of tissue injury in IFN-γ-deficient mice, but with 50% less induction than that in the wild-type[48], suggesting that the IFN-γ signal contributed half of the effect to induce MHC molecules triggered by tissue injury *in vivo*, while IFN-γ-independent signals were also at play. It is possible *in vivo* that the activated T cells secrete IFN-γ (Figure 1), which in turn induces more MHC molecules on APCs to facilitate antigen presentation to further accelerate T cells activation and IFN-γ secretion, thus forming a positive-feedback loop in the area of injury.

Cytosolic dsDNA-induced production of IL-1β and IL-18 is mediated by a rather independent upstream signal pathway that involves absent in melanoma 2, apoptosis-associated speck-like protein complex and caspase 1 cleavage[49-52] (Figure 1). Nevertheless, the contribution of a pro-inflammatory extracellular milieu to the development of autoimmunity should never be underestimated.

CONCLUSION

Numerous factors must be working together to trigger an autoimmune response: Environmental stimuli (*e.g.,* those that can cause tissue injury) and genetic predisposition (*e.g.,* having a specific human leukocyte antigen haplotype increases the risk of autoimmune diseases[53]). Studies have indicated that cytosolic naked dsDNA (either foreign or self orign) could be a universal factor that activates both innate and acquired immune responses in any tissue and cell type to trigger unfavorable immune responses in autoimmune-prone individuals.

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